ELSEVIER

Journal of Chromatography A, 785 (1997) 239–249

JOURNAL OF CHROMATOGRAPHY A

Review

Coupling chemical derivatization reactions with supercritical fluid extraction

Jennifer A. Field

Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331, USA

Abstract

Coupling chemical derivatization reactions with supercritical fluid extraction for the determination of trace levels of organic and organometallic compounds in liquid and solid matrices is reviewed. Derivatization is used to increase the solubility of analytes in supercritical carbon dioxide, to increase analyte volatility for gas chromatographic analysis and to integrate sample preparation steps in order to reduce analysis time and costs. Reactions that are covered in this review derivatize analytes possessing carboxyl, hydroxyl, sulfonic and amino groups to their alkyl, acyl and silyl derivatives. Derivatization is also used to derivatize active matrix sites to facilitate the release of analytes. Approaches used to couple derivatization with supercritical fluid extraction include reactions conducted prior to extraction, under in-site supercritical fluid conditions, or off-line under injection port conditions. Examples of applications are given for organic and organometallic compounds in environmental, pharmaceutical and agricultural product samples. © 1997 Elsevier Science B.V.

Keywords: Reviews; Derivatization; Supercritical fluid extraction; Silylation; Alkylation; Acylation; Organometallic compounds; Pesticides; Fatty acids; Phenols

Contents

0021-9673/97/$17.00
PII S0021-9673(97)00572-4

1. Introduction

The area of analytical scale extractions has received much attention recently because it is one of the least developed stages in the assemblage of steps taken to determine compounds in complex matrices. New techniques such as supercritical fluid extraction have developed over the last ten years in response to the need for more rapid and cost-effective methods that also reduce the amount of organic solvent discarded. As an extraction fluid, supercritical carbon dioxide has advantages including low toxicity, chemical inertness and ease of disposal. When used for extractions, supercritical carbon dioxide effectively combines analyte extraction, solvent evaporation, extract concentration and analyte collection into one or two steps.

Unfortunately, the use of relatively non-polar supercritical carbon dioxide is limited to the extraction of rather non-polar compounds, due to the limited solubility of polar compounds in supercritical carbon dioxide. Despite this limitation, one would like to take advantage of the properties of supercritical carbon dioxide for streamlining the typically time-consuming and tedious extraction procedures used to prepare polar analytes for analysis by gas chromatography. One approach that developed in response to the desire to extend the utility of supercritical fluids to polar compound analysis was the coupling of chemical derivatization reactions with supercritical fluid extraction. Derivatization reactions are used to decrease the polarity of polar analytes, which, in turn, increases their volatility, increases their solubility in supercritical fluids, promotes their separation from aqueous and solid samples, improves their detectability and enables the coupling of supercritical fluid extraction with various chromatographic methods of separation and detection.

In this review, the types of derivatization reactions, reagents and their limitations are discussed with respect to supercritical fluid extraction. Although derivatization reactions are used with high-performance liquid chromatography, they have received little attention. The two basic approaches in coupling derivatization with supercritical fluid extraction include derivatization of the analyte and derivatization of matrix active sites. Furthermore, applications that are covered in this review include (1) reactions performed prior to extraction that are used to improve the solubility and hence the recovery using supercritical fluids, (2) reactions that occur in-situ and (3) reactions where reagents are used to increase the solubility of analytes in supercritical fluids and subsequently derivatize the analyte under off-line (injection port) conditions. This review does not cover literature that describes the addition of derivatization reagents once an extraction has been performed [1–4] or for supercritical fluid chromatography applications [5]. Furthermore, this is not meant to be a comprehensive review of derivatization reactions, as an excellent review exists [6]. Rather, this review addresses the chemistry

and application of reactions that have been successfully coupled with supercritical fluid extraction.

2. Why derivatization reactions?

2.1. Volatility

Gas chromatography has the advantages of superior separation compared to liquid chromatography and a compatibility with a wide variety of detectors; However, volatility is the principal requirement for analyte analysis by gas chromatography. Polar organic compounds that contain active hydrogens (e.g. hydroxyl, amino and thiol groups) are typically of low volatility due to their tendency to self-associate or to associate with polar liquid or solid media through hydrogen bond formation. In order to increase polar analyte volatility, active hydrogens typically are masked by forming alkyl, silyl and acyl derivatives, which effectively reduces the tendency of polar analytes to form hydrogen bonds [6]. Derivatization also can be used to increase an analyte's thermal stability (e.g. organometallics [7]).

2.2. Reducing polarity and promoting separation

Reducing the polarity of analytes through chemical derivatization makes polar analytes more amenable to extraction by either conventional means (e.g. organic solvents) or by supercritical fluid extraction. Derivatization of active hydrogens decreases the reactivity of polar analytes with active sites of complex sample matrices and increases the solubility of polar analytes in non-polar fluids (e.g. organic solvents, supercritical fluids). Decreasing an analyte's polarity through derivatization reactions can be used to decrease the aqueous solubility of the compound, which, in turn, promotes the isolation of the compound from water using solid-phase extraction media, which can then be eluted with supercritical fluids.

2.3. Detection

Another important use of chemical derivatization reactions is to improve the detectability of compounds by detectors that are commonly used with gas chromatographs (e.g. flame ionization, electron capture and nitrogen–phosphorus detectors as well as mass spectrometers). Halogenated derivatives are used typically to promote the sensitive detection of analytes by electron capture detectors and negative chemical ionization mass spectrometry. Derivatization can alter ion fragmentation patterns, which can be used to elucidate compound structure and functional group composition [6]. Derivatization also can be used to enhance analyte detection using single wavelength ultraviolet, diode array, or fluorometric detectors, which are typically coupled with high-performance liquid chromatography.

2.4. Coupling with supercritical fluid extraction

Coupling derivatization reactions with supercritical fluid extraction combines the aforementioned advantages of supercritical fluid extraction with the ability of derivatization reactions to enhance polar analyte volatility, extractability and detection as well as to deactivate active matrix sites. Using derivatization reagents to modify supercritical fluids and to perform reactions reduces the consumption of organic solvents and reduces the number of preparative steps. Of interest are derivatization reagents that can be added directly to supercritical fluid extraction cells and that are tolerant to the presence of water, especially in the case of partially hydrated environmental or biological samples.

3. Derivatization reactions

Three principal types of derivatization reactions common to analytical chemistry have been coupled with supercritical fluid extraction including alkylation, silylation and acylation reactions. Each class of reactions replaces the active hydrogens of –OH, –NH and –SH groups. The reactions, mechanisms, catalysts and reagents typically used for derivatization reactions that have been coupled with supercritical fluid extraction are briefly described. For a more detailed description of the various types of derivatization reaction, see Knapp [6].

3.1. Alkylation

Alkylating reagents displace the active hydrogen in hydroxyl (–OH), carboxyl (–COOH), amine (NH), amide (COHN) and thiol (–SH) groups by nucleophilic displacement to form esters, ethers, thioethers, N-alkyl amines and N-alkyl amides, respectively. Alkylation reagents and their corresponding catalysts used in supercritical fluid extraction are listed in Table 1. Alkylation reagents include acidic methanol, alkyl halides such as methyl iodide and pentafluorobenzyl bromide (PFBBr), and tetraalkylammonium salts.

In the case of carboxylic acids, the use of acidic methanol protonates the keto groups of the carboxylic acid, which facilitates attack by methanol with the concomitant loss of water. Catalysts used with methanol include formic acid and alumina, which acts as a heterogeneous catalyst. Alkylation reactions involving alkyl halide reagents (e.g. methyl iodide and PFBBr) are promoted by either basic catalysts or anion-exchange resins. In base-catalyzed alkylation reactions involving PFBBr or methyl iodide, organic or inorganic bases deprotonate the functional group, thereby creating a better electrophile that can attack the nucleophilic alkylation reagent. Alkylation reagents used in this type of reaction typically have a good leaving group (e.g.

Table 1
Examples of reaction type, reagents and catalysts used to couple derivatization with supercritical fluid extraction.

Reaction type	Analyte	Reagent	Catalyst	Reference
Alkylation	Resin and fatty acids	Methanol	Formic acid	[29]
	Fatty acids	Methanol	Acidic alumina	[12]
	Fatty acids	PFBBr	Potassium carbonate	[12]
	Fatty acids, chlorophenoxy acids, phenols, sulphonamides	Tetraalkyl-ammonium salt	–	[13,14,25,26,33]
	chlorophenoxy acids	PFBBr	Potassium iodide	[32]
	Chlorophenoxy acids	PFBBr	Triethylamine	[13,30]
	Chlorophenoxy acids, benzimidazoles, thiouracils, phenols	Methyl iodide	Anion-exchange resin	[8]
	Chlorophenoxy acids	Methyl iodide	Tetraalkyl-ammonium salt	[13,28]
	Chlorophenoxy acids	BF_3–methanol	–	[14,25]
	Organotin compounds	Grignard reagents (RMgX)	–	[9,10]
	Organotin compounds	Sodium tetraethylborate	–	[7]
Silylation	Chlorophenoxy acids	HMDS/TMCS	–	[14]
	Dicarboxylic acids, alcohols	HMDS/TMCS	–	[23]
	Sterols	BSTFA/1% TMCS	–	[31]
Acylation	Phenols	Acetic anhydride	SAX/water	[19]
	Phenols		Triethylamine	[15–18]
	Phenols, β-blockers		Pyridine	[20–22]

BF_3, boron trifluoride.
BSTFA, N,O-bis(trimethylsilyl)trifluoroacetamide.
HMDS, hexamethyldisilazane.
PFBBr, pentafluorobenzyl bromide.
TMCS, trimethylchlorosilane.

bromide). Acidic analytes that are exchanged onto strong anion-exchange resins are considered to be in a "naked anion" form that readily reacts with alkyl halides (e.g. methyl iodide or PFBBr) to form their alkylated derivatives [8]. Note that hard acids and bases, such as HCl and NaOH, generally are not used as catalysts in supercritical fluid extraction, due to the potential for corrosion of stainless steel extraction cells and tubing.

Tetraalkylammonium salts serve several functions when used for derivatization reactions that are coupled with supercritical fluid extractions. First, tetraalkylammonium salts are phase transfer catalysts that facilitate the transfer of acidic analytes to non-polar phases through the formation of tetraalkylammonium ion pairs. Secondly, the tetraalkylammonium cation serves as a catalyst in alkylation reactions as described above. For these reasons, tetraalkylammonium salts aid in the solubilization and extraction of acid analytes using non-polar supercritical fluids, such as carbon dioxide. Thirdly, tetraalkylammonium ion-pairs reacts under high temperature conditions to form the alkylated derivative of an acid analyte and a volatile trialkylamine reaction by-product. Tetraalkylammonium ion-pair reactions occur during supercritical fluid extraction or under the high temperature conditions in the injection port of a gas chromatograph.

Because organometallic compounds, such as organotin compounds, are polar and non-volatile compounds in their underivatized states, derivatization is necessary to render them amenable to gas chromatographic analysis. In general, reactions for organometal compounds involve the nucleophilic attack of the metal ion (e.g. Sn) by the alkylation reagent. Grignard reagents that have been used to link organometal derivatization with supercritical fluid extraction include hexylmagnesium bromide [9] and ethylmagnesium chloride [10]. Grignard reagents have low solubility in supercritical carbon dioxide so that Grignard reactions are conducted prior to supercritical fluid extraction [7,9,10]. Sodium tetraethylborate, an alternative to Grignard reagents, was used to derivatize organotin compounds in aqueous solution [7] and to facilitate the isolation of organotin compounds from water using C_{18}-bonded phase silica Empore disks, with subsequent disk elution by supercritical carbon dioxide. Organoarsenic species were derivatized to their alkyl thioethers under in-situ supercritical fluid conditions with the addition of the reagent thioglycolic acid methyl ester [11].

The majority of alkylation reactions that have been coupled with supercritical fluid extraction are generally tolerant to the presence of water. In the case of acidic methanol conditions, water is formed as a reaction by-product [12] and sodium tetraethylborate is used as an aqueous alkylation reagent for organometallics [7]. Alkyl halides and tetraalkylammonium reagents were used to extract and react with herbicides in soils of measurable moisture contents [13]. On the other hand, Grignard reagents require anhydrous conditions so that samples must be dried or the organometallic compounds must be extracted into an organic phase prior to reaction. Of the alkylation reactions that have been coupled with supercritical fluid extraction, the two examples that used PFBBr together with potassium salt catalysts included clean-up steps. Where the tetraalkylammonium reagents are used as injection port derivatization reagents, reagent removal is not advised as it is detrimental to reaction efficiency!

3.2. Silylation

The reactivity of silylation reagents towards active hydrogens decreases in the order of hydroxyl (aliphatic>phenolic>carboxyl), amine, to amide. For this reason, silylation reactions primarily are used in supercritical fluid extraction to derivatize aliphatic and aromatic hydroxyl groups, although some examples of silyl derivatives of carboxylic acids were reported (Table 1).

Silylation reactions involve the nucleophilic attack by the analyte heteroatom (O, N or S) on the Si atom of the silylation reagent. Silylation reagents must have a good leaving group in order to stabilize the loss of a silyl group and have little tendency to compete with the derivatized group of the analyte in a back reaction. Although a range of silylation reagents are available, hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) are conducting silylation reactions under in-situ supercritical fluid conditions. Silylation reagents are often used in combinations because silylating reagents have varying abilities to donate silyl groups and

because reagents such as TMCS can influence the silyl donor strength of other silylation reagents [6].

Generally, silylation reactions are thought to require relatively anhydrous conditions, so that soils with moisture contents greater than 0.4% may reduce reaction efficiencies and analyte recovery [14]. Silylation reagents typically do not need to be removed prior to injection; however, Knapp [6] notes that excess silylation reagents can foul flame ionization detectors.

3.3. Acylation

Acetylation reactions that use acetic anhydride are the most common type of acylation reactions that are coupled with supercritical fluid extraction. Typically, organic bases such as triethylamine and pyridine are used to catalyze the reaction and to accept the acetic acid formed as a reaction by-product. Strong anion-exchange resins can also be used as a catalyst, but not as an acid receptor. Although fluorinated acylation reagents are available, they have received little attention for use with supercritical fluid extraction.

The presence of water is not an issue for some acetylation reactions as acetylation can be performed in aqueous solutions with the direct addition of acetic anhydride to water samples. Although Knapp [6] recommends that the excess acetic acid formed during the reaction should be removed to prevent adverse effects on the gas chromatography (GC) column, only reactions conducted using triethylamine as the catalyst/acid receptor under supercritical fluid conditions were cleaned up to remove excess acetic acid [15–18]. By comparison, reactions using pyridine as the base or where a strong anion-exchange resin was used as a solid support received no clean-up prior to analysis [19–22].

4. Conceptual and experimental approaches

4.1. Analyte vs. active matrix site derivatization

By far the majority of methods that combine derivatization with supercritical fluid extraction focus on analyte derivatization. As discussed in previous sections, alkylation, silylation and acylation reactions are used to address the problems of polar analytes, including poor volatility, separation and detection. Others have suggested that derivatization reactions may play a role in matrix deactivation [23] and at least one report used silylation reagents to deactivate the sample matrix in order to improve the recovery of analytes that do not undergo derivatization (polynuclear aromatic hydrocarbons) [24].

4.2. In-situ and off-line derivatization

4.2.1. Pre-extraction derivatization

Derivatization reactions can be used to enhance the extractability of polar analytes whether reactions are performed prior to, during, or after supercritical fluid extraction. When performed prior to extraction, derivatization decreases the polarity of analytes and increases their solubility in supercritical fluids, thus improving analyte recovery [7,9,10,19]. In the case of the Grignard reaction for the derivatization of organometals, the reagent ethylmagnesium chloride has a low solubility in supercritical carbon dioxide, so the reaction must be conducted prior to supercritical fluid extraction [7,9,10]. Derivatization was also performed in order to decrease the polarity of phenols and organotin compounds so that they are readily isolated from water using hydrophobic sorbents that can then be eluted with supercritical fluids [7,25].

4.2.2. In-situ derivatization

The majority of methods that couple derivatization with supercritical fluid extraction were conducted under in-situ supercritical fluid conditions. The benefits of conducting reactions in-situ includes reducing sample handling, decreasing the total number of preparative steps and obtaining extracts that can be directly analyzed by GC. In addition to participating in reactions, reagents can act as modifiers during the supercritical fluid extraction step and enhance the solubility of analytes or interact with matrix sites. Generally, reactions that were coupled with supercritical fluid extraction did not require the removal of excess reagents (Table 1). In one case, the supercritical effluent of an extraction cell in which fatty acid methyl esters were formed in-situ was transferred directly to gas chromatographs for on-line analysis [12]. Reactions conducted under in-situ supercritical fluid conditions typically have static extraction

periods ranging from 5 to 30 min followed by a dynamic extraction period of generally 10–30 up to 60 min.

4.2.3. Post-extraction (injection port) reactions

Tetraalkyl(aryl)ammonium reagents are reported to derivatize analytes under in-situ supercritical fluid conditions under the high temperature conditions of a gas chromatograph injection port [13,14,26,27]. Although some disagreement exists over whether chlorophenoxy acid herbicides are derivatized in-situ or under injection port conditions [13,14,25], tetraalkylammonium alkylation reagents clearly play important roles both in the extraction and derivatization of acidic analytes, regardless of where derivatization occurs. Because tetraalkylammonium reagents are injection port derivatization reagents, reagent removal is unnecessary prior to injection.

5. Applications

The development of applications coupling derivatization reactions with supercritical fluid extraction was motivated by the need for alternative methods for the determination of polar compounds in order to address problems in environmental science, pharmaceutics, agriculture, nutrition and toxicology. Although the areas of application are varied, the analytical problems associated with polar compound analysis are similar. In all cases, sample matrices are complex and contain trace-to-high levels of the analytes of interest. For sample matrices ranging from sediment to urine to seeds, water typically is present and its effect on the reaction and extraction conditions must be considered.

5.1. Sediment and soil

Much attention has focused on the recovery of trace levels of polar organic contaminants from sediment and soil. Sediments and soils represent very complex matrices with many potential interferences that can be co-extracted and derivatized. Because interferences can consume reagent, excess quantities of reagent typically are added for the extraction of sediments and soils.

5.1.1. Chlorophenoxy acid herbicides

Chlorophenoxy acids are the subject of great interest due to the tedious nature of the conventional methods for extracting chlorophenoxy acid herbicides from soil and sediment. Tetraalkylammonium salts were first used to facilitate the extraction and derivatization of 2,4-dichlorophenoxy acid (2,4-D) and dicamba from sediment under supercritical fluid conditions [25]. A total of 30 min was required to recover >90% of native and spiked 2,4-D and dicamba from river sediment and soil. Variability in chlorophenoxy acid recovery is evident when tetraalkyl(aryl)ammonium salts are used with sediment and soil. As an example, tetraalkylammonium salts recovered only 14–19% of spiked chlorophenoxy acid in 30 min [14] while 50% or less was recovered on addition of trimethylphenylammonium salt (TMPA) and other alkyl(aryl)ammonium salts [13]. Other types of derivatization reaction have been evaluated since for the recovery of 2,4-D and other chlorophenoxy acid herbicides from soil. For example, 60–70% of 2,4-D and 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) were recovered from spiked soil in 30 min using methyl iodide and an alkylammonium salt as the catalyst [28]. Alkylation with methanol and BF_3 as catalyst recovered 90% of spiked 2,4-D in 30 min using supercritical carbon dioxide [29]. Although the reaction and extraction conditions were not optimized, silylation recovered 31% of spiked 2,4-D in 75 min [14]. In another survey, chlorophenoxy acids were recovered (46–93% with R.S.D. values of 3–6%) from soil using ion-pair catalyzed alkylation with methyl iodide as the reagent, and 23–80% was recovered using PFBBr with triethylamine as the catalyst [13]. An inter-laboratory comparison using PFBBr as the alkylation reagent and triethylamine as the catalyst recovered <70 to 150% of eleven chlorophenoxy acids spiked onto soil in 60–90 min [30].

5.1.2. Fatty and other polar acids

Resin and fatty acid concentrations in sediment, determined using methanol–formic acid as the reagent under supercritical fluid conditions were 250% of the those obtained by conventional Soxhlet extraction. Poorer recovery (35–45%) was achieved using PFBBr and K_2CO_3 as the catalyst [29]. Qualitative information was obtained on the fatty

acid (and other polar acids) composition of marine sediment by in-situ silylation [23].

5.1.3. Phenols

In-situ acetylation reactions gave good recoveries (>85%) of phenols from sediment in 35 min, with good agreement with values obtained by Soxhlet extraction and steam distillation [15]. The recovery of phenol from soil by in-situ acetylation was 81±6% of the reported certified values [21,22]. Pentachlorophenol was recovered from spiked soil using in-situ acetylation in 10 min with recoveries of 87–100% and a 6.2% R.S.D. [16]. The values obtained by the in-situ acetylation approach were in good agreement with values obtained using steam distillation and were better than those achieved using supercritical carbon dioxide and water as the modifier. Concentrations of pentachlorophenol determined using the in-situ acetylation procedure were higher than the reported values for reference soils.

5.1.4. Organometals

Tributyl and phenyltin compounds were recovered (106–111%) from sediment in 20 min when coupled with a pre-extraction Grignard reaction. Poorer recovery with achieved for mono- and dibutyltin compounds [9]. Recoveries of organoarsenic compounds spiked onto soil ranged from 90–103% with R.S.D. values of 1–8%, using thioglycolic acid methyl ester as the reagent under in-situ supercritical fluid conditions [11].

5.2. Sewage sludge

Sterols determined in sewage sludge using a 30-min in-situ silylation and extraction procedure gave similar results to that obtained by ultrasonic extraction with methylene chloride–acetone [31]. With the addition of a tetraalkylammonium salt, sulfonated aliphatic surfactants were quantitatively extracted in their ion-pair form from sewage sludge in 15 min under supercritical fluid conditions. The precision of the method indicated by the R.S.D. was 5%. In the case of sulfonated surfactants, derivatization occurred under the high temperature conditions of the gas chromatograph's injection port.

5.3. Aqueous samples using solid supports

Chlorophenoxy acid herbicides spiked onto C_{18} resin were recovered as their pentafluorobenzyl derivatives using potassium carbonate as the catalyst [32]. By manipulating the density of the supercritical carbon dioxide, excess pentafluorobenzyl bromide was separated from the derivatized analyte in an on-line mode prior to gas chromatographic analysis.

Chlorophenoxy acid herbicides, pentachlorophenol and acidic N-containing pharmaceutical chemicals (benzimidazole, sulfamidine and thiouracil drugs) were first exchanged onto strong anion-exchange resin and then simultaneously derivatized to their methyl esters and eluted from the resin using methyl iodide as the alkylation reagent under supercritical fluid conditions [8]. Recoveries of 92–99% for the chlorophenoxy acid herbicides and of 78% for pentachlorophenol were obtained within 40 min, while lower (15–60%) recoveries were obtained for the pharmaceutical analytes. Acetylation reactions involving acetic anhydride and pyridine as the catalyst recovered 60–70% of aryloxypropanolamines (β-blockers) in 45 min of extraction from C_{18} disks that were used to isolate the analytes from urine [20].

Phenols leached from wood soot were isolated onto C_{18} disks and quantitatively eluted as their methyl esters using TMPA in a total of 15 min [25]. Phenols in distilled water were isolated onto strong anion-exchange disks and eluted as their acetylated derivatives under supercritical fluid conditions; a total of 18 min was needed to recover 75–87% of the phenols with a R.S.D. of 4% [19]. By comparison, recovery was 100% when phenols were acetylated first in aqueous solution, isolated onto a styrenedivinylbenzene disk, and recovered with supercritical carbon dioxide [19].

For the extraction of organotin compounds from water, derivatization reactions are used primarily to promote the isolation of organotins from water onto hydrophobic sorbents (C_{18}-bonded phase silica disks) and to increase their solubility in supercritical fluids so that supercritical carbon dioxide can be used to recover the derivatized analytes from the C_{18} disks. Organotin compounds were determined in sea water by first extracting the di- and tributyltin compounds onto a C_{18} disk and then performing a

Grignard reaction just prior to extraction with supercritical carbon dioxide; recoveries of 92–102% were obtained with R.S.D. values of 6.6–8.2% [10]. In a second approach, butyltin and phenyltin compounds were derivatized to their ethyl esters in aqueous solution, enriched on a C_{18} disk, and then eluted from the disk with supercritical carbon dioxide [7]. Recoveries ranged from 79–115% with R.S.D. values ranging from 3–11%.

5.4. Microbiological samples

Tetraalkylammonium salts were employed as ion-pair/injection port reagents for the determination of fatty acids in microorganisms (Table 2) [25,33]). Lyophilized whole cells extracted using TMPA under supercritical fluid conditions gave fatty acid concentrations and compositions that were in good agreement with those obtained by conventional chloroform–methanol–water extractions [25]. Using a similar approach, ten species of bacteria were differentiated based on their fatty acid compositions [33]. In both cases, a total of 30 min and <5 ml of organic solvent were required.

5.5. Agricultural products

In one of the first examples of reactions coupled with supercritical fluid extraction, polar acids and alcohols were determined in coffee beans and tea by conducting silylation reactions under supercritical fluid conditions (Table 2) [23]. The fatty acid content of single oil seeds (soybeans, evening primrose and peanut) was determined by performing in-situ alkylation with methanol and alumina, which had been treated with methanol and acted as a

Table 2
Compound classes, their derivatives, and sample matrices

Compound class	Derivative	Matrix	Reference
Chlorophenoxy acids	Alkyl	Soil	[13,14,25,28,30]
		SAX disk/water	[8]
		C_{18} disk (spiked)	[32]
		Rice	[28]
		Extrelut (urine/water)	[28]
	Silyl	Sand	[14]
Fatty acids	Alkyl	Microorganisms	[25,33]
		Sediments	[29]
		Oil seeds	[12]
Dicarboxylic acids and alcohols	Silyl	Sediments, coffee beans, tea	[23]
Phenols	Acyl	Soil/sediment	[15,16,21,22]
		Leather	[17,18]
		SAX disk (water)	[19]
	Alkyl	C_{18} disk (wastewater)	[25]
Sterols	Silyl	Sewage sludge	[31]
Sulfonic acids	Alkyl	Sewage sludge	[27]
Sulphonamides	Alkyl	Diatomaceous earth, silica gel	[28]
Aryloxypropanolamines (β-blockers)	Acyl	C_{18} disk (urine)	[20]
Thiouracils, benzimidazoles, sulfamidine	Alkyl	SAX disk (water)	[8]
Organoarsenic compounds	Thio ether	Sand	[11]
Organotin compounds	Alkyl	Sediment, C_{18} disk (seawater)	[7,9,10]

heterogeneous catalyst (Table 2) [12]. The restrictor from the supercritical fluid extraction was used to transfer derivatized analytes directly to the inlet of a gas chromatograph. Good agreement was obtained on the fatty acid composition between the derivatization/supercritical fluid extraction method and the conventional Soxhlet extraction method. Methyl iodide and a tetraalkylammonium ion-pair reagent catalyst were used to recover 80–90% of chlorophenoxy acid herbicides spiked onto rice [28].

5.6. Materials

A method was developed and refined to measure the amount of pentachlorophenol residues in processed leather using derivatization coupled with supercritical fluid extraction [17,18]. In this case, method development was motivated by an interest in estimating the potential human exposure to pentachlorophenol, which is used as a preservative and fungicide in the leather industry. The concentrations of pentachlorophenol obtained using acetic anhydride and triethylamine in 40 min of in-situ supercritical fluid derivatization were comparable to those obtained by an 8-h Soxhlet extraction procedure.

6. Summary

Combining derivatization with supercritical fluid extraction first emerged in 1990 as an alternative approach for the determination of polar compounds in complex solid matrices. Since that time, a number of applications have been developed for solid, liquid and biological samples that save time and minimize sample preparation compared to conventional extraction and derivatization methods. A wide array of polar compounds, including drugs, fatty acids, acid herbicides, phenols, organometals and surfactants are determined using methods where derivatization occurs either prior to extraction, under in-situ conditions, or after extraction under injection port conditions. The validity of these alternative approaches is illustrated by the number of applications where good agreement is obtained between concentrations obtained by derivatization/supercritical fluid extraction and by conventional extraction and derivatization methods. Additional research is needed to better understand the factors affecting reaction efficiency and their effect on recovery, because variable recoveries have been reported for analytes in similar matrices using a similar type of reaction. With the emergence of subcritical water extraction as an alternative to supercritical fluid extraction (see chapter by Louie in this volume), clear opportunities exist for combining derivatization reactions with aqueous extractions.

7. Abbreviations

BF_3	Boron trifluoride
BSTFA	N,O-Bis(trimethylsilyl)trifluoroacetamide
C_{18}	Octadecyl-bonded silica
COOH	Carboxyl group
2,4-D	2,4-Dichlorophenoxy acetic acid
2,4,5-T	2,4,5-Trichlorophenoxy acetic acid
HCl	Hydrogen chloride
HMDS	Hexamethyldisilazane
OH	Hydroxyl group
NaOH	Sodium hydroxide
NH	Amine group
PFBBr	Pentafluorobenzyl bromide
SAX	Strong anion exchange
SH	Thiol group
TMCS	Trimethylchlorosilane
TMPA	Trimethylphenylammonium salt

References

[1] J.M. Wong, Q.X. Li, B.D. Hammock, J.N. Seiber, J. Agric. Food Chem. 39 (1991) 1802.
[2] A. Izquierdo, M.T. Tena, M.D. Luque de Castro, M. Valcárcel, Chromatographia 42 (1996) 206.
[3] M.T. Tena, M.D. Luque de Castro, M. Valcárcel, Anal. Chem. 57 (1995) 1054.
[4] H. Emeteborg, E. Björklund, F. Ödman, L. Karlsson, L. Mathiason, W. Frech, D.C. Baxter, Analyst 121 (1996) 19.
[5] L.A. Cole, J.G. Dorsey, T.L. Chester, Analyst 116 (1991) 1287.
[6] D.R. Knapp, Handbook of Analytical Derivatization Reactions, Wiley, New York, 1979.
[7] Y. Cai, J.M. Bayona, J. Chromatogr. Sci. 33 (1995) 89.
[8] S.N. Chatfeld, M.Y. Croft, T. Dang, E.J. Murby, G.Y.F. Yu, R.J. Wells, Anal. Chem. 67 (1995) 945.
[9] Y. Cai, R. Alzaga, J.M. Bayona, Anal. Chem. 66 (1994) 1161.

[10] R. Alzaga, J.M. Bayona, J. Chromatogr. A 655 (1993) 51.
[11] B.W. Wenclawiak, M. Krah, Fresenius' J. Anal. Chem. 351 (1995) 134.
[12] J.W. King, J.E. France, J.M. Snyder, Fresenius' J. Anal Chem. 344 (1992) 474.
[13] V. Lopez-Avila, N.S. Dodhiwala, W.F. Beckert, J. Agric. Food Chem. 41 (1993) 2038.
[14] E.A. Rochette, J.B. Harsh, H.H. Hill Jr., Talanta 40 (1993) 147.
[15] H.-B. Lee, T.E. Peart, R.L. Hong-You, J. Chromatogr. 636 (1993) 263.
[16] H.-B. Lee, T.E. Peart, R.L. Hong-You, J. Chromatogr. 605 (1992) 109.
[17] A. Meyer, W. Kleiböhmer, J. High Resolut. Chromatogr. 19 (1996) 267.
[18] A. Meyer, W. Kleiböhmer, J. Chromatogr. A 718 (1995) 131.
[19] P.H.-T. Tang, J.S. Ho, J. High Resolut. Chromatogr. 17 (1994) 509.
[20] K. Hartonen, M.-L. Riekkola, J. Chromatogr. B 676 (1996) 45.
[21] M.P. Llompart, R.A. Lorenzo, R. Cela, J. Chromatogr. Sci. 34 (1996) 43.
[22] M.P. Llompart, R.A. Lorenzo, R. Cela, J. Microcol. Sep. 8 (1996) 163.
[23] J.W. Hills, H.H. Hill, T. Maeda, Anal. Chem. 63 (1991) 2152.
[24] J.W. Hills, H.H. Hill, J. Chromatogr. Sci. 31 (1993) 6.
[25] S.B. Hawthorne, D.J. Miller, D.E. Nivens, D.C. White, Anal. Chem. 64 (1992) 405.
[26] M.T. Tena, M.D. Luque de Castro, M. Valcárcel, Chromatographia 40 (1995) 197.
[27] J.A. Field, D.J. Miller, T.M. Field, S.B. Hawthorne, W. Giger, Anal. Chem. 64 (1992) 3161.
[28] M.Y. Croft, E.J. Murby, R.J. Wells, Anal. Chem. 66 (1994) 4459.
[29] H.-B. Lee, T.E. Peart, J. Chromatogr. 594 (1992) 309.
[30] V. Lopez-Avila, J. Benedicto and W.F. Beckert, Herbicide Metabolites in Surface Water, ACS Symposium Series 630, American Chemical Society, Washington, D.C., 1996, pp. 63–76.
[31] D.-K. Ngygen, A. Bruchet, P. Arpino, Environ. Sci. Technol. 29 (1995) 1686.
[32] R. Hillmann, K. Bachmann, J. High Resolut. Chromatogr. 17 (1994) 350.
[33] A.A. Gharaibeh, K.J. Voorhees, Anal. Chem. 68 (1996) 2805.